

# Equine Science Update

Monthly reports from the world of equine research

October 2001

## Possible new treatment for Equine Cushing`s Disease

Recent work suggests that a drug used to treat human Cushing`s disease may be beneficial in the management of the disease in horses.

Equine Cushing`s Disease (ECD) is associated with excessive cortisol production by the adrenal gland. In almost all cases it is caused by a functional tumour ("adenoma") of the *pars intermedia* of the pituitary gland.

In normal horses, ACTH from the pituitary stimulates the release of cortisol from the adrenal glands. The blood cortisol level is controlled by a complex "feedback" mechanism. Basically, an increase in blood cortisol inhibits further ACTH release, which in turn causes the cortisol level to fall.

In horses with Cushing`s Disease the pituitary adenoma produces ACTH, and other similar molecules, some of which increase the "potency" of ACTH. In addition, the adenoma does not respond to the usual feedback controls. The signs associated with ECD are mostly due to the increased cortisol activity.

Drugs such as bromocriptine (Pergolide®) or cyproheptadine (Periactin®) have been used to control the disease. These drugs act on the pituitary gland to reduce ACTH production.

A different approach has been investigated recently using trilostane (Modrenal®), Stegram Pharmaceuticals, UK), a drug which blocks cortisol production within the adrenal gland itself.

Researchers at the Royal Veterinary College, led by Dr Cathy McGowan, carried out a trial to evaluate the value of the drug in the management of ECD. Twenty horses and ponies were included in the study. Most were ponies; there were twelve mares and eight geldings. Each animal showed at least three signs of Cushing`s disease - eg hirsutism (long curly coat) (20/20 cases), lethargy (19/20), laminitis (16/20),

excessive drinking/urinating (11/20). The diagnosis of ECD was confirmed using the combined dexamethasone suppression / TRH stimulation test.

The horses were treated with trilostane (at doses between 0.4 and 1mg/kg) by mouth once a day. The response to treatment was assessed after 30 days and after 12 months.

"After 30 days treatment all horses showed an improvement in at least 1 of the clinical signs. All horses were less lethargic. Thirteen cases (81%) showed an improvement or no recurrence of laminitis. However, three severe cases of laminitis did not improve." Dr McGowan reports "The eleven cases which had been drinking and urinating excessively, all showed a decrease towards normal. These improvements continued over the 12 months of the study. The treatment had no effect on the hirsutism - the horses remained hairy."

Three horses which were given the lower dose (0.4mg/kg) developed laminitis during the trial. The dose was increased to 1mg/kg and no further recurrence occurred.

Treatment with trilostane did not affect the results of the dexamethasone suppression test. But the response to the TRH stimulation test was more normal.

"The treatment seemed safe and efficacious. The best effect was seen at the 1mg/kg dose rate. We have not noticed any side effects."

Dr McGowan concludes "This is a useful therapy for the treatment of ECD, especially with respect to improving the quality of life. We think it merits further study"

source: British Equine Veterinary Association Congress 2001.

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## Benefits of feeding multiple forages.

Recent research suggests that providing stabled horses with more than one type of forage, may reduce the risk of straw impaction colic.

Feral horses eat a diet consisting of a wide variety of grasses, herbs and other plants. In contrast, most stabled horses are fed hay as their main source of forage. The restricted access to pasture that many horses experience may be associated with stereotypic behaviour such as crib-biting and weaving.

Debbie Goodwin, Nell Davidson and Pat Harris conducted a study to see if there was a difference in behaviour between horses fed a single type of forage and those fed several different forages. Twelve horses in a competition Dressage and Eventing yard were used in the trial. They were routinely stabled except for a maximum of four hours at pasture.

Two identical stables were used in the trial. Each was 12 feet square, bedded with straw, and contained two water buckets. One stable contained a single hay net of high quality meadow hay. The other, in addition to the hay, contained five proprietary forages.

Horses were introduced to each of the two boxes on two occasions and their behaviour was recorded over a period of five minutes, using a wall mounted video camera. The researchers then measured the time spent on feeding and other activities by examining the recordings.

All six forages were eaten. Hay was the least popular forage. Horses spent more time foraging in the multiple forage stable than they did in the stable in which only hay was provided. The difference, however, was not statistically significant. Horses spent significantly more time eating the straw bedding in the single forage stable. They also showed significantly more searching behaviour in the single forage box (ie they spent more time moving and looking out of the box).

The authors conclude that, in this short term study, providing multiple forages reduced both

the amount of straw eaten and the time spent on behaviour which suggested frustration.

"The horses appeared to enjoy having a choice of forages" said Dr Pat Harris. "If these effects persist over longer periods, providing more than one forage could reduce the incidence of straw impaction colic in stabled horses, and provide other welfare benefits through enrichment of the environment."

for more details see:

D Goodwin, H P B Davidson, P Harris. Behavioural enrichment for stabled horses using multiple forages. Proceedings of the Third International Congress on Veterinary Behavioural Medicine. 2001

## Research request:

### **Trial of herbal treatment for equine Cushing's disease.**

Robert Eustace at The Laminitis Clinic is investigating the use of aqueous extract of Chaste Berry (*Vitex agnus castus*) for the treatment of equine Cushing's disease.

He is interested in horses which have either not received any previous medication for Cushing's disease or which seem intolerant of other drug treatments.

More details are available on the website <http://www.equilife.co.uk/herbaltrial.html>

Owners with suitable horses and ponies can apply to joint the trial.

Apply to the Laminitis Trust

by e-mail to [rae@equilife.co.uk](mailto:rae@equilife.co.uk)

or by post to The Laminitis Trust, Mead House Farm, Dauntsey, Chippenham, Wilts. SN15 4JA

or by telephoning 0870-444-0676.

## Progressive laryngeal paralysis.

Recurrent laryngeal neuropathy (RLN), also known as idiopathic laryngeal hemiplegia (ILH), is by far the most common cause of laryngeal dysfunction in horses. It causes partial or total paralysis of the muscles which open the larynx during inspiration. Affected horses make a characteristic noise when breathing in ("whistling" or "roaring") and are less able to perform strenuous work. Opinions vary as to whether the condition is present from birth or is acquired early in life, but it is usually first detected when the horse starts training. Most authorities agree that, once present, the condition does not deteriorate further.

Evidence was revealed at the World Equine Airways Symposium recently to challenge that view. Prof Dixon and his team at the Royal (Dick) School of Veterinary Studies, Edinburgh, presented the results of a study which showed that RLN can develop and progress in adult horses. They found that laryngeal function can deteriorate, sometimes slowly and progressively, but sometimes suddenly and dramatically.

They examined the records of 351 confirmed cases of RLN in older horses of mixed breeds. In 52 cases (about 15%) there was evidence that laryngeal paralysis had progressed.

All horses were examined using an endoscope on at least on occasion. The degree of laryngeal paralysis was graded on a scale of 0 (normal) to 5 (total paralysis).

In 30 cases there was both endoscopic and clinical evidence that the condition had progressed. These horses had been examined initially, and over the course of the clinical deterioration. Seventeen of these horses were normal (grade 0 or 1) on first examination. On subsequent examination the RLN had deteriorated by an average of 3 grades.

In the remaining 22 cases, endoscopy was only carried out after the deterioration had occurred. There was, therefore, only clinical evidence of progression. This included the sudden onset of respiratory "noises" (16 cases) or such sounds getting worse (6 cases) and associated poor

performance. When they examined these cases endoscopically they found that 13 of the 22 horses had marked RLN (grade 4 or 5). It was apparent from the performance history of these horses that the condition had only recently developed.

They quote one notable example in a 6 year old National Hunt racehorse. The horse had an excellent racing record, with no history of respiratory "noises". Over a 5 month period it had developed progressively more noise and increasing exercise intolerance. Endoscopic examination showed total paralysis (grade 5) which was incompatible with the previous racing record. The horse could not have achieved its racing success if the laryngeal paralysis had been present at the time.

According to Prof Dixon, these findings are particularly relevant in the context of examinations for purchase. "In three of the cases the recorded deterioration occurred within 10 weeks of purchase, and in 2 cases it was the source of conflict between purchaser and vendor."

"It should be considered that differences in clinical and endoscopic findings between vets who perform examinations before and after purchase, perhaps just weeks apart, may simply reflect clinical progression of RLN, rather than inter-assessor variation or, has been alleged in some such cases, negligence by the vet who performed the pre-purchase examination."

The findings are also relevant when considering the management of less severe cases of RLN. These are often treated by being "Hobday-ed", a relatively simple procedure. However, Prof. Dixon warns that if such cases appear to suffer from progressive RLN, it may be better to treat them by laryngoplasty ("tie-back") initially, rather than have to subject them to a second general anaesthetic and surgery if the Hobday operation is not sufficient.

Source: World Equine Airways Symposium, Edinburgh. 2001

## Bacterial role in laminitis?

A better understanding of the mechanisms involved in the development of laminitis has been provided by work carried out in Australia. Dr Pollitt and his colleagues working at the University of Queensland have shown that bacterial exotoxins play a key role.

It is known that laminitis due to excessive carbohydrate ingestion is associated with several related changes in the hindgut of the horse. The gut contents become more acidic, mostly due to an increase in lactic acid. Many of the normal bacterial flora cannot survive in such conditions, and they die, releasing toxins that are present in their cell walls ("endotoxins").

Bacteria which thrive in such acidic conditions include Gram-positive cocci such as *Streptococcus bovis*, which grows rapidly and soon becomes the dominant bacterium.

The increase in endotoxins in the gut and their absorption into the blood has been recognised in horses with laminitis for some time. However, experiments have shown that endotoxin is not responsible for laminitis.

Recently attention has turned to the involvement of streptococcal exotoxins in the production of laminitis. It has been shown that horses can survive carbohydrate overload without developing laminitis if *Streptococcus bovis* overgrowth is prevented by feeding the antibiotic virginiamycin.

Previous Work by Dr Chris Pollitt and others has shown that a group of enzymes called matrix metalloproteinases (MMP's), especially gelatinase A and B, play an important role in laminitis by breaking down major structural components of the basement membrane. This leads to disruption of the attachment between the basement membrane and the epidermis. (Pollitt & Daradka 1998)

More recently Dr Pollitt has been investigating whether bacterial toxins can activate MMP's and play a part in causing laminitis.

He has used isolated sections of laminar hoof tissue ("explants") which extend from the inner

hoof wall through the lamellar junction to the dermal connective tissue. He incubates them with bacterial broth cultures, or purified bacterial toxins.

Using this technique, Dr Pollitt has been able to demonstrate that *Streptococcus bovis*, three other species of Gram-positive streptococci and 3 gram-negative bacteria all activate MMPs. The MMPs are then able to break down the attachment between the basement membrane and the laminar epidermal cells and cause lamellar explants to separate.

Two bacterial exotoxins, thermolysin (a bacterial metalloprotease) and SPeB (streptococcal pyrogenic exotoxin B) both induce lamellar separation but only thermolysin does so by activation of MMPs.

"We propose that the rapid increase in streptococcal species in the caecum and colon in response to favourable changes in the intestinal environment (induced by carbohydrate overload) may cause laminitis via the production of exotoxins capable of activating MMP's within the laminae. Once activated, these MMPs can degrade important parts of the basement membrane leading to separation of the basement membrane from the epidermal cells."

"The results of this study provide a unifying aetiological mechanism for the development of carbohydrate-induced equine laminitis" said Dr Pollitt. "We have demonstrated that bacterial exotoxins have a key role in the development of equine laminitis. If we can identify the specific exotoxins involved we may be able to develop an effective prophylactic treatment for equine laminitis."

ref: CC Pollitt , M Daradka . (1988) Equine laminitis basement membrane pathology: loss of type IV collagen, type VII collagen and laminin immunostaining. *Equine Vet. J.* 26 (Suppl)139-144

Source: **In vitro evidence for a bacterial pathogenesis of equine laminitis.**

*Veterinary Microbiology* (2001) 79, 209-223  
BA Mungall, M Kyaw-Tanner, C C Pollitt.

## DNA Typing for parentage testing

DNA "fingerprinting" has been behind the investigation of what were believed to be the remains of Tsar Nicholas and his family, the study of mammoths in the tundra permafrost and Egyptian mummies. The technology is now being used for identity testing of horses.

Mike Scott is head of the Genetic Services department at the Animal Health Trust. Most of his work involves parentage testing of horses. He outlined the use of DNA typing to a recent meeting of the Association of Racecourse Veterinary Surgeons.

He explained that the test is based on a knowledge of genetics. "The horse has 64 (32 pairs) of chromosomes in the nucleus of each cell. One of each pair of chromosomes is derived from each parent. The test works on the principle that any DNA present in the foal must have come from one or other of the parents".

Although DNA is an almost infinitely complex molecule, in one sense its structure is simple in that it is made up of 4 nucleotides: adenine, guanine, cytosine and thymine (A, G, C and T). Each gene comprises a short section of the DNA chain. The order in which the nucleotides occur determines which proteins the genes will make. A difference of one single nucleotide is enough to change the protein that a gene will produce.

Less than 10% of all the DNA serves to code for genes. The rest is "junk", some of which is called "satellite DNA". The original work with DNA fingerprinting used "mini-satellites" - areas of repeated sequences of DNA. Current technology uses "micro-satellites" - regions where small groups of nucleotides are repeated.

The number of repeats varies between individuals, and it is this variation which is measured. The DNA profile (and the blood type) remains the same throughout the life of the horse.

Polymerase Chain Reaction (PCR) technology has revolutionised all aspects of molecular biology, including DNA typing. This technique permits the amplification of the particular region of the DNA to be examined. Because only very small amounts of DNA exist within the cells, it is necessary to "amplify" it so that enough is

available to run the test. By the time the PCR has cycled 30 times there has been a 1 billion-fold increase in the amount of DNA. At the same time fluorescent dyes are added. The mixture is processed in the machine which separates the DNA into bands and presents the results in a visual form.

To carry out the test a source of DNA is required. Only a small amount is needed. For example, 3 hair roots would provide enough DNA using the PCR amplification process. Currently a blood sample is used and DNA is obtained from the white blood cells. Originally it had been hoped to be able to use the DNA in hair follicles. Unfortunately, in foals the hair tends to snarl and leave the root which contains the DNA.

### Comparison with blood typing

The older system of blood-typing looked for blood groups (ie particular proteins which had been produced under instructions carried by the DNA).

### Advantages of DNA typing:

- lower costs because it is more automated. (The PCR technique is patented - so royalties have to be paid each time the test is used.)
- greater efficacy. DNA (microsatellite) typing is expected to solve more than 99.9% of cases of disputed parentage. In comparison, the older blood typing technique would have solved about 97% of cases.
- hair can be used instead of blood.
- it is better for genetics research.

One disadvantage of DNA typing compared with blood typing is the length of time taken to complete the test. With blood typing it was possible to take a sample from a horse at the sales and confirm its identity the same day. That is not the case with DNA typing which takes longer.

Unfortunately blood typing and DNA typing are incompatible. That is, the results of one cannot be "translated" to the other. This means that there will have to be a total change from the old to the new system.

Source: Association of Racecourse Veterinary Surgeons meeting 2001

## External nasal dilator strips - do they work?

Investigations at the University of Illinois have found no benefit in using external nasal dilator strips in horses.

External nasal dilator strips have been used by human athletes to aid breathing during exercise. A similar product is now available for horses, which claims to reduce nasal resistance to airflow and reduce the work of breathing. It has been suggested that it might be helpful in reducing the incidence of exercise-induced pulmonary haemorrhage (EIPH) and improve exercise-induced changes in blood oxygen and carbon dioxide levels.

In a study by Prof Gordon Baker and his team at the University of Illinois, seven healthy and sound Thoroughbred horses were worked on a treadmill running at maximum speed. Each horse was exercised on a treadmill running at maximum speed on two occasions a week apart. Each horse wore a nasal dilator strip on one of the two exercise sessions.

Measurements were taken at rest, during moderate exercise, during near-maximal exercise and during recovery. Parameters recorded included core temperature, arterial and venous blood gases, blood lactate and ammonia concentrations.

The results showed no difference between treated and untreated horses. Exercise caused the plasma ammonia and blood lactate to increase significantly regardless of whether the horses were wearing the nasal strip. Changes in oxygen and carbon dioxide levels were similar in both treatment groups. All horses, in both groups, had EIPH.

Prof Baker concluded "Our data indicated that application of an external nasal dilator strip neither improved the exercise-induced hypoxaemia and hypercapnia. Nor did it diminish anaerobic metabolism or the incidence of EIPH in Thoroughbred horses performing strenuous exercise".

source: British Equine Veterinary Congress, Harrogate. 2001.

## Dorsal Displacement of the Soft Palate : treatment of by thermal cautery.

Dorsal Displacement of the Soft Palate (DDSP) occurs in racing Thoroughbreds during strenuous exercise. It is believed to be a common cause of respiratory distress and poor performance. Affected horses often make a characteristic "gurgling" sound, but up to 30% of cases of DDSP make no noise. DDSP is probably a neuromuscular dysfunction leading to collapse of the nasopharynx. As it is a functional rather than a physical problem, it is impossible to confirm the diagnosis without the use of

upper airway endoscopy performed during galloping to a level of fatigue on a high-speed treadmill.

There are many alleged reasons for the condition. Consequently a range of treatments exist. Management practices which are used to help prevent DDSP include ensuring an adequate level of fitness, avoiding discomfort in the mouth, and using tongue straps or dropped nose bands.

Various surgical techniques have been claimed to be effective in treating DDSP including: removing a strip of tissue at the back of the soft palate ("staphylectomy"), cutting the "strap muscles" on the underside of the neck; enlarging the epiglottis with Teflon®; removal of a longitudinal strip of mucosa from the oral surface of the soft palate ("Ahern tension palatoplasty") and endoscopic laser cautery of the soft palate in the standing horse.

Bob Ordidge, a veterinary surgeon in Yorkshire uses a technique of thermal cautery (firing) which he has found to be as effective as, and to have advantages over, other surgical techniques. Between January 1988 and September 2000 he treated 232 cases which had a history of abnormal respiratory noise and/or poor performance suggestive of "gurgling". They had no upper respiratory tract abnormalities on resting endoscopy, and no sign of respiratory infection.

The horses ranged in age from 2 - 11 years. Most were geldings (157). There were 50 mares and 25 colts. They were categorized by type of work : flat racing (77); hurdling (60); chasing (37); dual flat racing and hurdling (32); dual hurdling and chasing (26).

#### **Technique**

Before treatment the horse's mouth was thoroughly washed out. Short-acting anaesthetic agents were used which gave surgical anaesthesia for about 20 minutes. This was sufficient to carry out the procedure.

Custom built "irons" made of 1cm steel rods, 50cm long, with a 4-6cm "T" at one end were used to cauterize the soft palate, starting at the junction with the hard palate.

The aim of the cautery is to obliterate the two para-sagittal folds. Although access to the back of the soft palate is limited, Mr Ordidge does not find it is necessary to go all the way back. "Recent studies of horses exercising on treadmills have shown that DDSP starts with

billowing of the front of the soft palate" he points out.

#### **Post-operative management.**

The horses were fed soft food until normal appetite returned after 12-48 hours. Antibiotics were given for 3 days. Non steroidal anti-inflammatories were given for short-term pain relief as needed.

The horses were walked and trotted for 4 weeks and then returned to cantering. "Some horses actually returned to racing within 30 days."

#### **Results**

Two hundred and nineteen horses raced following treatment, 13 horses did not race afterwards for unrelated reasons. The treatment was considered to be successful in about 75% of horse according to the trainers. Almost 50% stopped gurgling, a further 25% made less noise than before the operation. Two horses were said to be worse. Analysis of the racing form of the horses showed that 51.67% improved after treatment; 45.93% stayed the same and 2.39% got worse.

#### **Advantages of the technique**

Mr Ordidge explains that the success rate with this procedure is comparable to other methods. "Thermal cautery of the oral surface of the soft palate is a simple, quick and low cost procedure. It only requires a short time out of training, causes minimal adverse effects and is not clinically detectable. It probably works by causing the soft palate to become stiffer and less influenced by upper airway pressure changes."

source: World Equine Airways Symposium, Edinburgh. 2001

## Ultrasound diagnosis of small colon strangulation

In the treatment of colic it is vital that those conditions which require surgical correction are identified early. Appropriate treatment can then be provided rapidly maximising the chance of a successful outcome. Strangulation of the small colon is particularly difficult to diagnose as the usual signs of intestinal compromise such as pain, changes in heart rate and intestinal sounds are only mild. Signs of colic in such cases are often slow to develop and only deteriorate gradually.

Dr Freeman and her colleagues have used transrectal ultrasonography as an aid to diagnosis of small colon strangulation. They describe two cases which were referred to the Royal Veterinary College Equine Hospital. The first animal, a 12 year old Welsh cob, had shown low grade abdominal pain for two days. The second, a 20 year old Arab cross pony had sudden onset moderate abdominal pain. In both cases, the clinical parameters such as heart rate, mucous membranes and intestinal sounds did not indicate a strangulating lesion.

On rectal examination the main feature was distension of the small colon with food material and gas, and a build up of gas in the large colon. Conditions that they considered as possible diagnoses included simple obstruction by impacted faecal material (impaction), obstruction by a mass within the intestine (such as a foreign body or an enterolith) and colitis.

However, when they examined the small colon wall by ultrasound scan, they found that the small colon wall was thickened (10-15mm thick in the first case; and 8-10mm in the

second.) "Other work that we have done has shown that the normal small colon wall is about 3-4 mm thick" said Dr Freeman. "It has a five layered appearance of alternating hyper-echoic (lighter) and hypo-echoic (darker) layers. In these two horses we found that the five layered appearance of the small colon was distorted. All layers were increased in echogenicity."

On the basis of the ultrasound findings, both cases underwent exploratory surgery. In both, the small colon was found to be strangulated by a pedunculated lipoma. They removed the strangulated intestine and the lipoma, and performed an end-to-end anastomosis of the remaining intestine.

"The use of transrectal ultrasonography provided a sensitive method of detecting a compromised small colon, and allowed prompt and effective surgical intervention" said Dr Freeman. "We suggest that transrectal ultrasonography is used in cases where small colon pathology is suspected, to assist in differentiating surgical from non-surgical conditions".

for more details see:

### Use of transrectal ultrasonography to aid diagnosis of small colon strangulation in two horses.

S L Freeman, J C Boswell, R K W Smith  
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